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Patentanmeldung Nr. Patent application No. Demande de brevet n°

03078086.0

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Rhenium (I) complexes of nucleo-purines

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A61K51/00

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PT RO SE SI SK TR LI

RHENIUM(I) COMPLEXES OF NUCLEO-PURINES

The invention relates to rhenium complexes of nucleopurines, which can be used as radio-therapeutic Cisplatin analogs.

The mononuclear, octahedral $^{186}Re(I)$ and/or $^{188}Re(I)$ complexes disclosed are able to interact with purine residues in the DNA of tumor cells to form 1,2-intrastrand adducts in a manner similar to that of Cisplatin. These compounds might be a better alternative for radioisotope therapy or chemotherapy of cancer. If applied with stable isotopes of rhenium, the compounds might exhibit a cytotoxic effect similar to that of cis-Pt. Each single type of interaction or the combination of both, radiation and functional interaction with DNA, can lead to a significantly increased therapeutic index.

Although cisplatin is a very effective anticancer drug, its undesirable side effects as well as inherent and acquired resistance reduce its clinical efficacy. These limitations, combined with the extraordinary success of Cisplatin and closely related second and third generation platinum antitumor agents, have stimulated the search for new inorganic complexes having cytotoxic properties. However, clinical application of other transition metal chemoterapeutic and radiopharmaceutical agents has been slow.

DNA is the major biological target of platinum compounds and their toxicity is correlated with the formation of 1,2-intrastrand adducts between the N7 atom of two adjacent purine residues. Biologically active platinum compounds are usually characterized by the presence of two *cis*-anionic labile ligands which are displaced prior to the formation of 1,2-intrastrand adducts. In the development of therapeutic antitumor agents the *cis*-dilabile-ligand metal (*cis*-DLLM) motif may prove to be an important starting point. It might also be desirable to employ compounds that might function mechanistically as cisplatin causing intrastrand linkages to DNA, in combination with an inherent radioactivity of the metal center. Such class of compound would act to inhibit DNA transcription while delivering a highly localized radiation dose in the target tumor tissues.

The aim of the present invention is to design a transition-metal complex which would combine both properties.

Rhenium(I) alkoxo and hydroxo carbonyl complexes have recently shown to be very potent inhibitors of growth in suspended tumor L1210 lymphoid leukemia cells and other types of human tumor cell lines. These complexes inhibit DNA synthesis by inhibiting dihydrofolate reductase and other enzymes for purine and pyrimidine pathways. Interaction with nucleo-purines in a fashion similar to that of Cisplatin has not been ruled out. It might be that these compounds may bind to the nitrogenous bases after displacement of the alkoxide or hydroxide ligands.

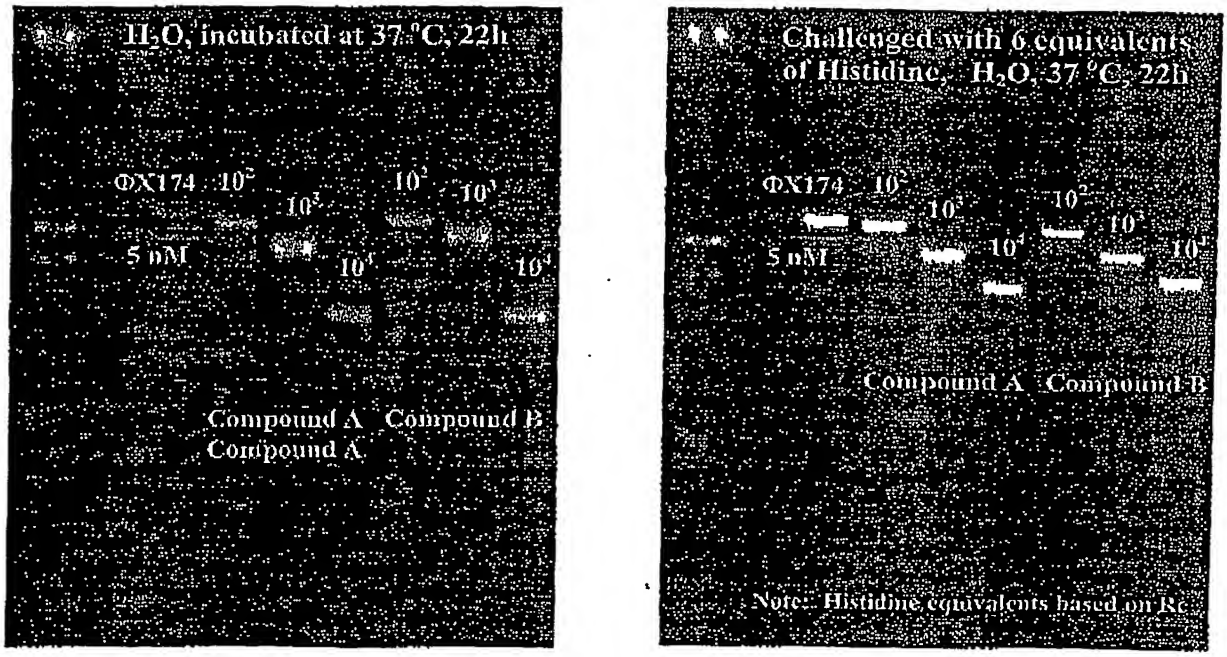
The inventors believe that a mononuclear octrahedral ^{186}Re(I) or ^{188}Re(I) complex may combine the inherent radioactivity of the metal center with the mechanistic properties of Cisplatin. They have in fact studied the interaction of a rhenium(I) compound containing the *fac*-$[M(CO)_3]^+$ moicty and the *cis*-DLLM motif with guanosine (**G**) and 2-deoxyguanosine (**2dG**). They are able to present evidence that two nucleo-purines bind the Re(I) center and do so at a rate comparable to that of platinum compounds.

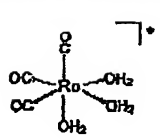
Compounds of formula $Re(G)(CO)_3Br\cdot\frac{1}{2}G$ and $Re(2dG)_2(CO)_3Br$ have been synthesized by treatment of $[Re(H_2O)_3(CO)_3]^+$ or $[Re(Br)_3(CO)_3]^{2-}$ with two equivalents of the heterocyclic N-donor base in methanol.

The present invention thus relates to the new rhenium tricarbonyl compounds as described in the following examples and their use in radiotherapy.

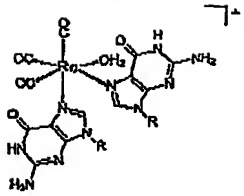
EXAMPLE 1

1. Evidence that $[Re(CO)_3]^1$ is able to induce structural changes when bound to FX174 circular DNA (Figure 1, left) and it is not released when challenged with Histidine (Figure 1, Right).





Compound A



Compound B

Note: The exponential numbers appearing on the bands correspond to the molar equivalents of Re compound added to the plasmid. In particular 10^2 correspond to one Re molecule every 55 base pairs (bp), 10^3 one Re molecule every 5.5 bp and 10^4 two Re molecules every bp.

Figure 1

EXAMPLE 2

2. Evidence that $[Re(CO)_3]^+$ exhibits cytotoxic properties.

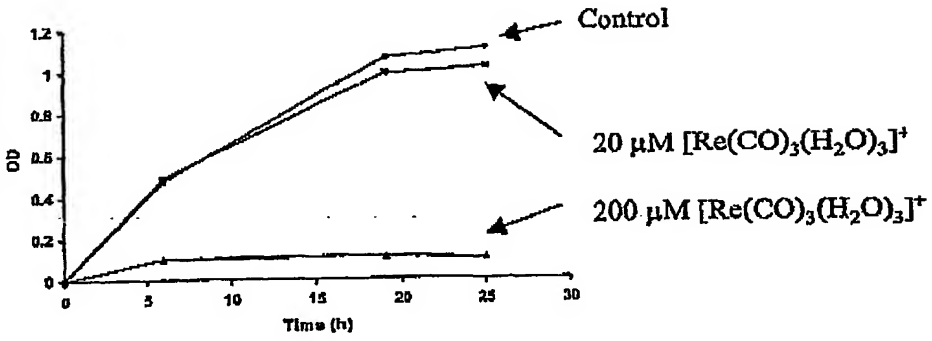


Figure 2 Melanoma Breast Cancer cell Proliferation Assay.

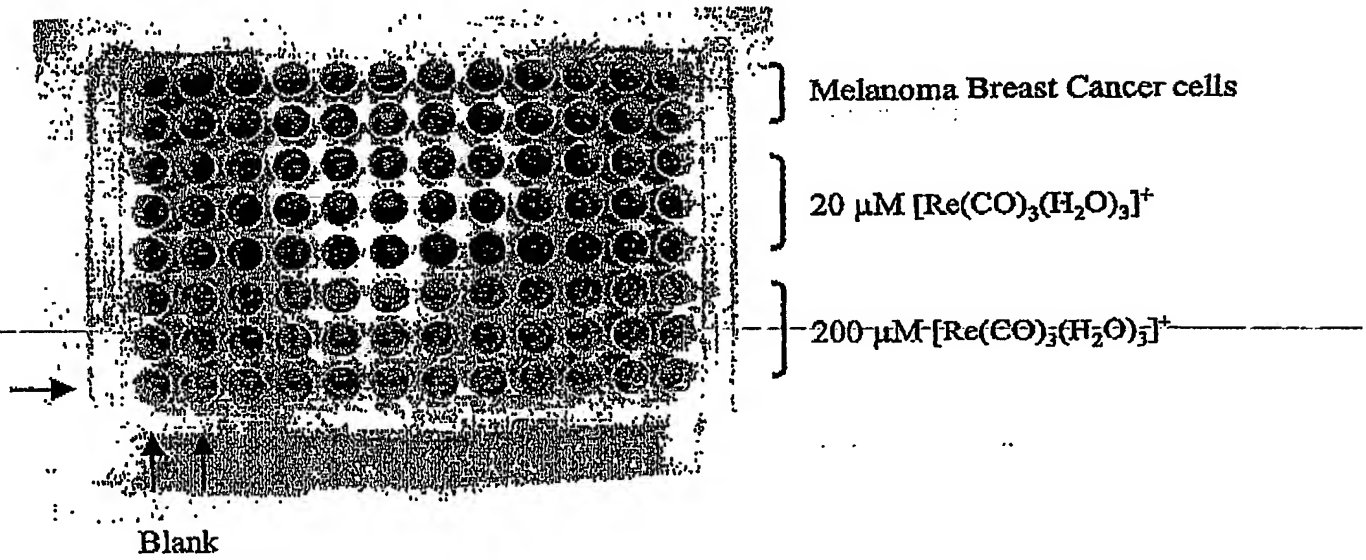
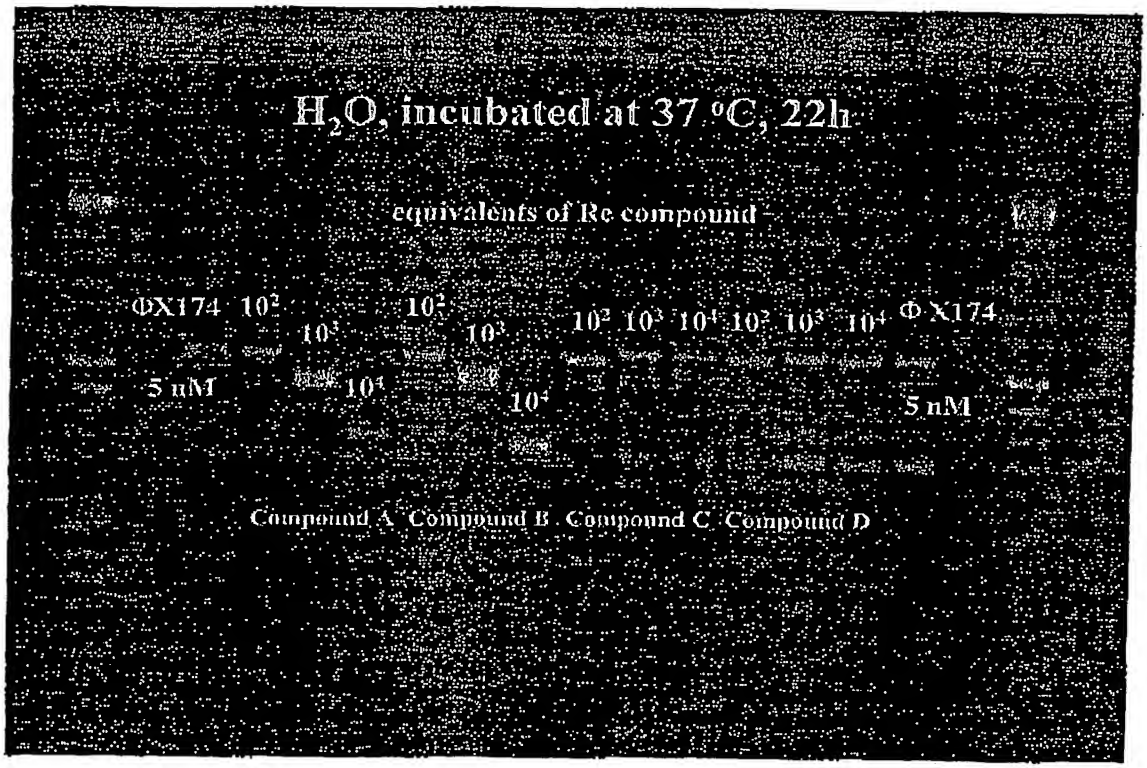
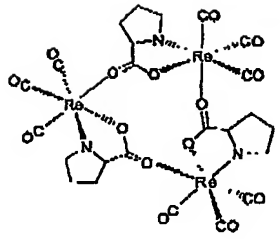


Figure 3 Melanoma Breast Cancer cell Proliferation Assay.

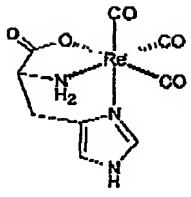
EXAMPLE 3

3. Evidence that two *cis*-labile ligands are required to induce the structural changes in FX174 circular DNA.





Compound C



Compound D

Note: Compound C has been shown to bind one base under similar conditions (see Figure 5).

Figure 4

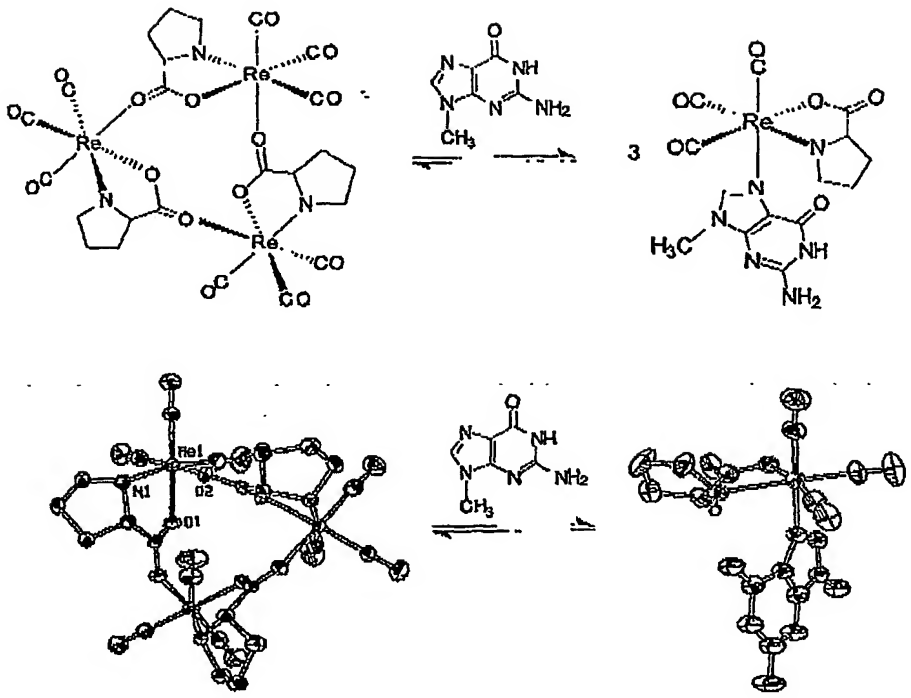
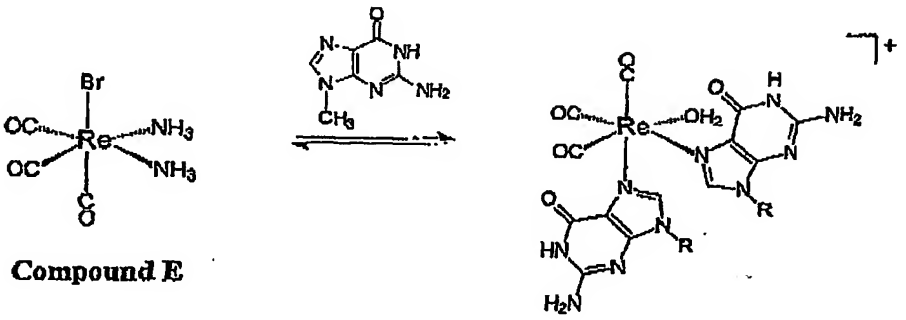


Figure 5 Reaction of Compound C with a DNA base and relative crystal structures.

EXAMPLE 4

4. Evidence that the $[Re(CO)_3]^1$ core can be protected by labile ligands (natural or artificial) which are replaced by DNA bases (see Figure 6).



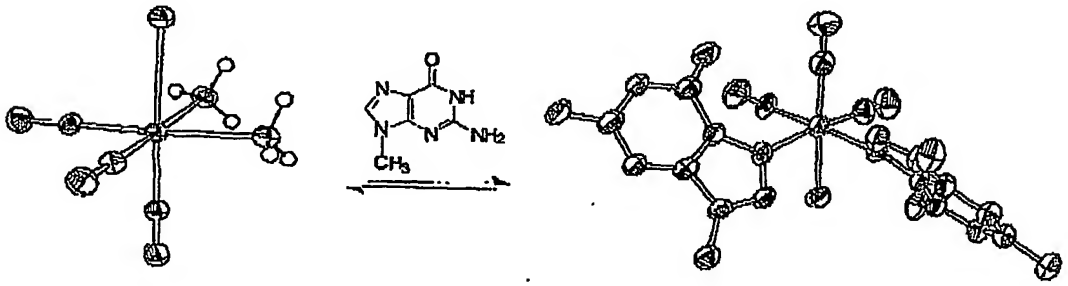


Figure 6 Reaction of Compound E with a DNA base and relative crystal structures.

EXAMPLE 5

5. Evidence that the $[Re(CO)_3]^+$ core induces similar structural changes in F X174 circular DNA as cisplatin (Figure 7).

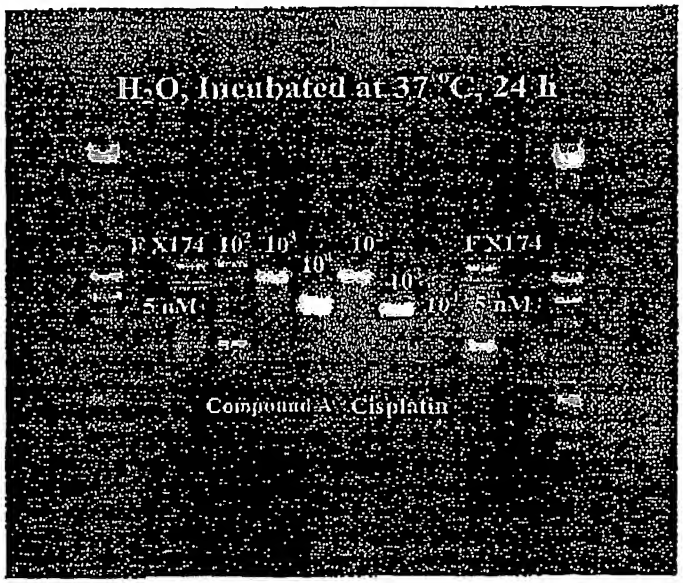
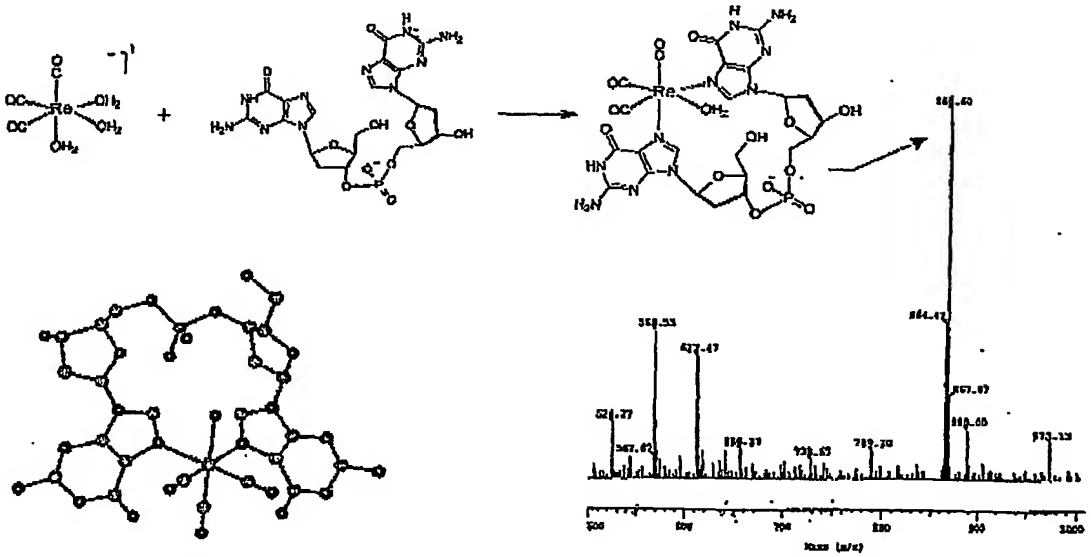


Figure 7

EXAMPLE 6

6. Evidence that the $[Re(CO)_3]^+$ core can bind to oligonucleotides comprising a GpG motif.



Insight II Optimized Structure

MS Spectrum of $[Re(CO)_3(GpG)]^+$

Figure 8 Evidence of binding of Compound A to GpG.

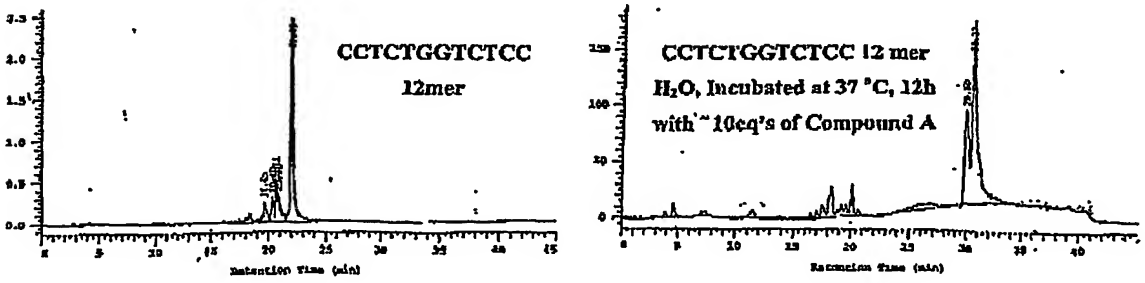


Figure 9 Chromatogram showing the effect of Compound A on CCTCTGGTCTCC double stranded dodecamer.

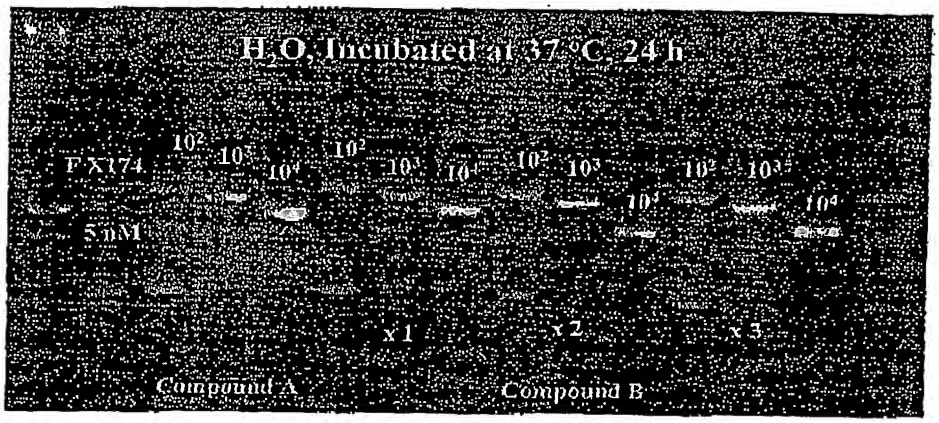
CONCLUSIONS REGARDING EXAMPLES 1-6:

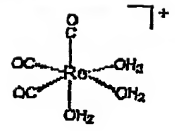
Based on the experimental evidence it is concluded:

1. that the $[Re(CO)_3]^+$ core can bind oligonucleotides comprising a GG motif with good stability.
2. that the $[Re(CO)_3]^+$ core can cause similar DNA structural changes as cisplatin.
3. that the results are unexpected since coordination to DNA bases of the above mentioned core should result in sterically too crowded complexes to have good stability.
4. that the $[Re(CO)_3]^+$ core surrounded by a proper set of ligands, can yield a complex with the following advantages:
   - I. Can combine radio- and chemotoxic characteristics; a double feature which is a major advantage over cisplatin.
   - II. Allows at the same time chemotherapeutic and diagnostic action.
   - III. Can easily be combined with vectors (i.e. polypeptides) that allow targeting, active uptake and degradation in the cytoplasm.
   - IV. Contrary to most other strategies with result in the design of Re based compounds exclusively suited for radiotherapeutic purposes where the metal core is prevented from interacting further at the target site, these complexes can, upon delivery, actively participate in the biochemistry at the desired target tumor site.

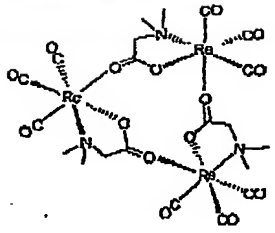
EXAMPLE 7

F X174 DNA Experiments. I have repeated the experiments with $[Re(NNdiMeGly)]_3$ and I got a better picture. Note how the effect of $[Re(NNdiMeGly)]_3$ on F X174 DNA is similar to ReAA.





Compound A



Compound B

Figure 10

The scheme below depicts the reaction of [Re(NNdiMeGly)]$_3$ with 9-MeG and it was deduced from HPLC-MS experiments.

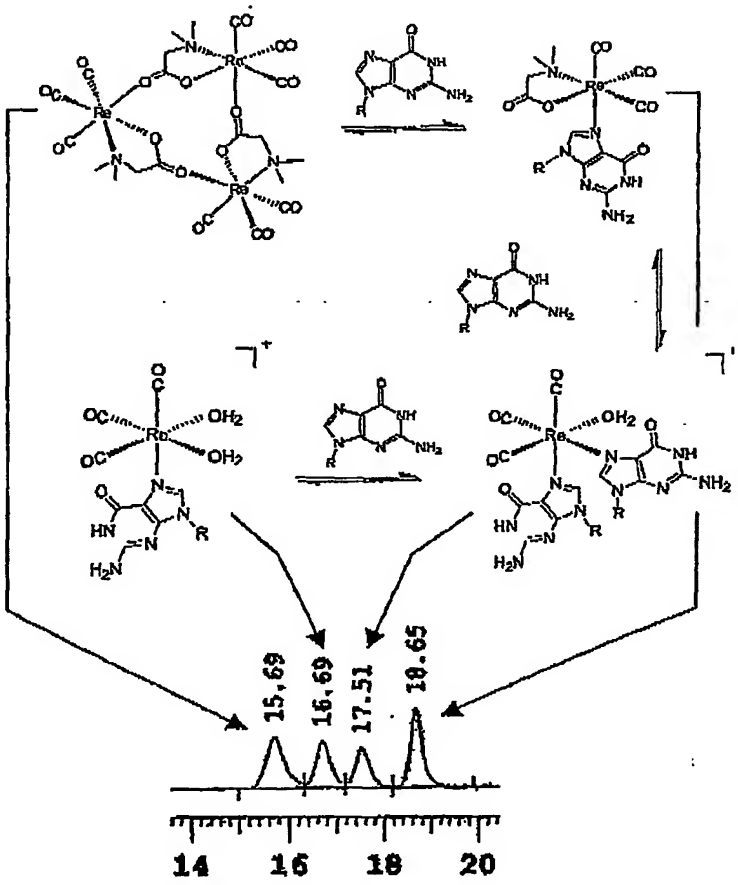


Figure 11

EXAMPLE 8

Effects of Re complexes on the proliferation of breast, ovarian and gastric cancer cell lines. All the experiments (i.e. cell proliferation) were checked at three different complex concentration: 50, 100 and 200 μM.

1. MDA line (breast cancer cells)

From the graph shown in figure 12 it follows that ReAA, Re(9MeG)2 and (RePro)3 all appear (with varying degree of success) to stop the proliferation of this cell line; ReHis has little or no effect.

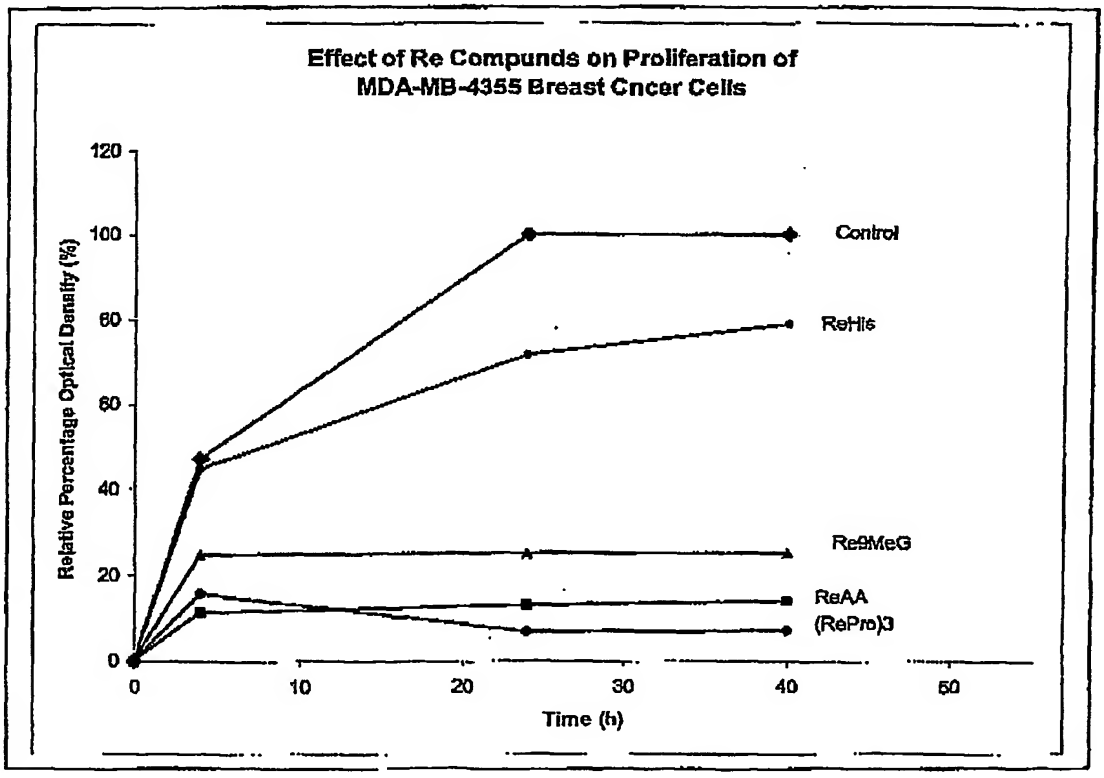


Figure 12

The surprising result in this case is the activity of (RePro)3 which shows a strong antitumor activity against MDA cancer cells.

2. OVMZ (ovarian cancer)

The experiments (i.e .cell proliferation) were checked at three different complex concentration: 50, 100 and 200 μM. Only the highest concentration showed significant deviation from the control and all the graphs you see are at 200 μM of the Re compound. As in the MDA case Ree AA shows a strong antitumor activity (see figure 13) followed by (RePro)3. Re(9MeG)2 and ReHis show little or no activity.

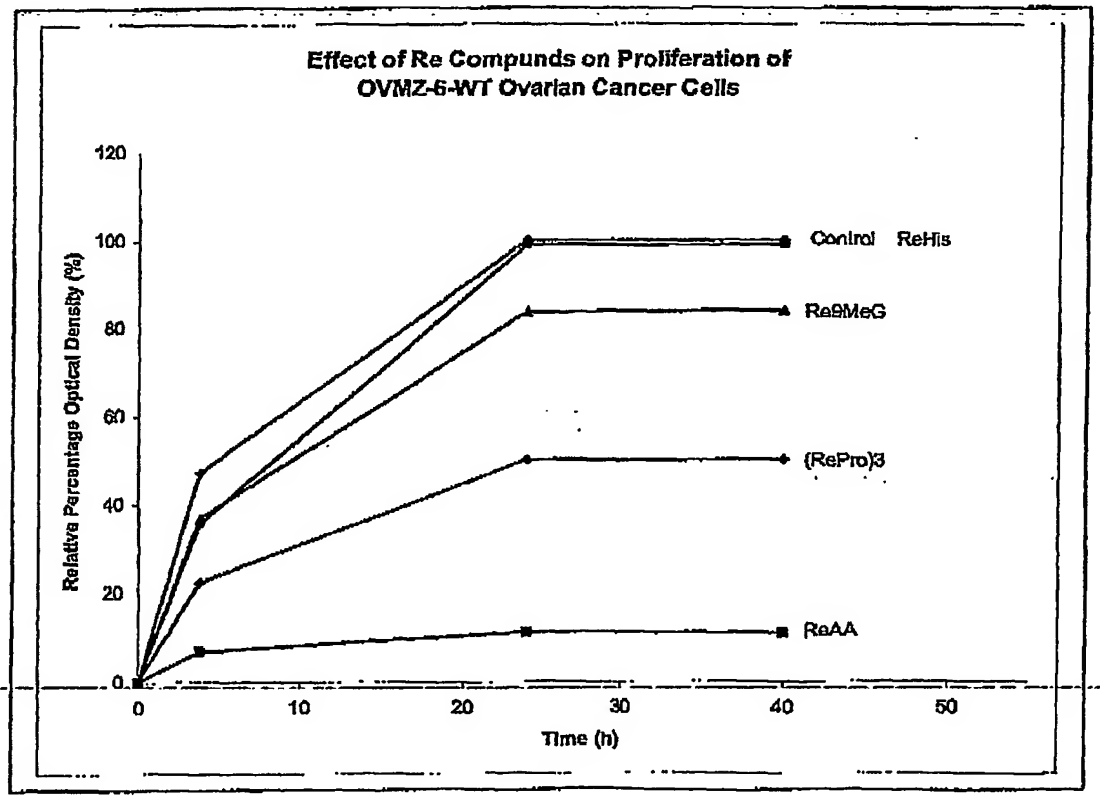


Figure 13

3. HSC (gastric cancer)

The last cell line against which we have checked the activity of the Re complexes is HSC (gastric cancer). These cells have the peculiarity that they have almost a double number of chromosomes, 70 instead of 46 normally present in human cells. In this case all compound but ReHis show little or no activity (see figure 14).

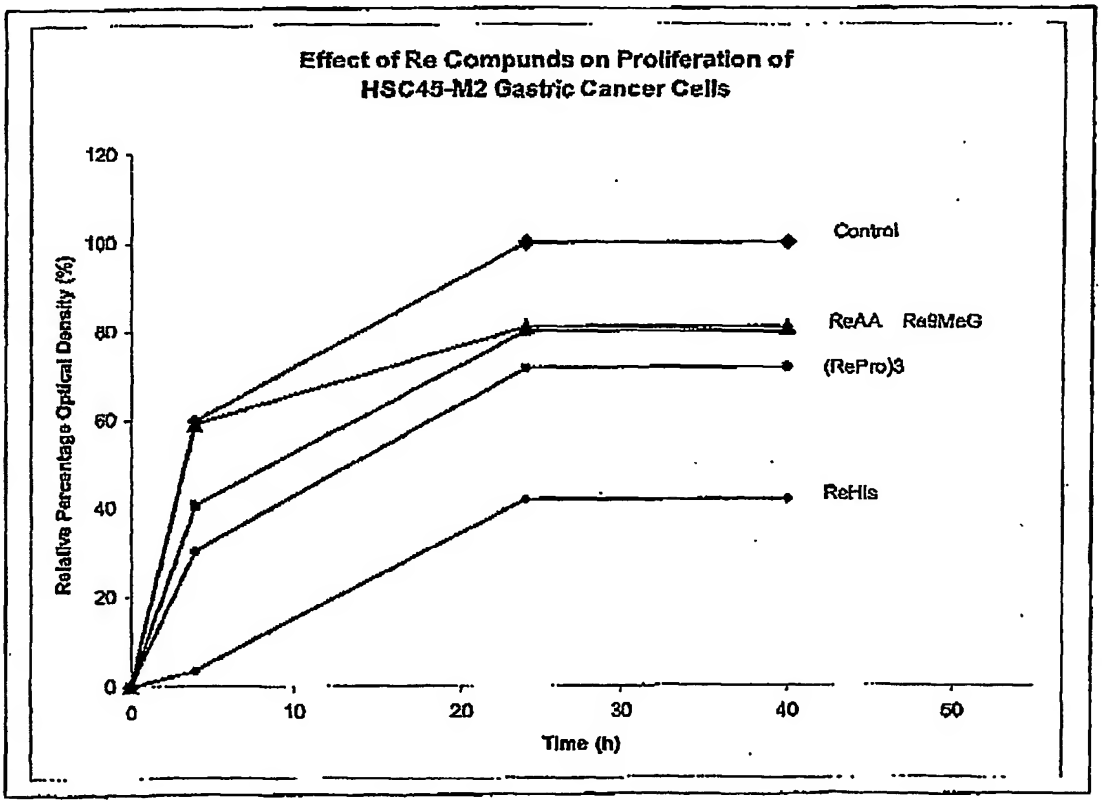
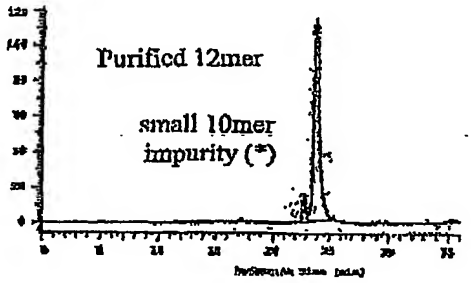


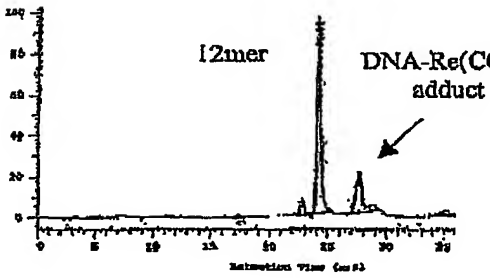
Figure 14

EXAMPLE 9
12mer Experiments.

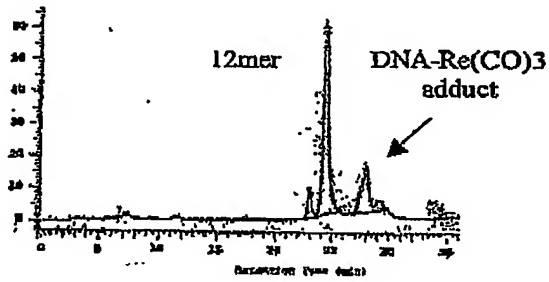
First the dodecamer (HPLC 1) was purified and then it was reacted with one equivalent of ReAA. HPLC 2 shows the result of the reaction after incubation in H_2O at 37°C, 24h. We can clearly see an adduct being formed. After a 48h incubation period there is not much change in the chromatogram (HPLC 3). An equilibrium has been reached.



HPLC 1

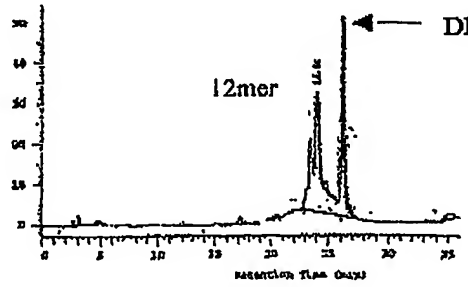


HPLC 2, 24h



HPLC 3, 48h

The same reaction was tried with cisplatin. First $[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ was made from $[Pt(NH_3)_2Cl_2]$ and $AgNO_3$ so to obtain the most reactive form of cisplatin. HPLC 4 shows the result of the reaction after incubation in H_2O at 37°C, 24h. One clearly sees the DNA-cisplatin adduct and it is clear that at least half the DNA has reacted. In the case of ReAA about 15% DNA has reacted.



HPLC 4

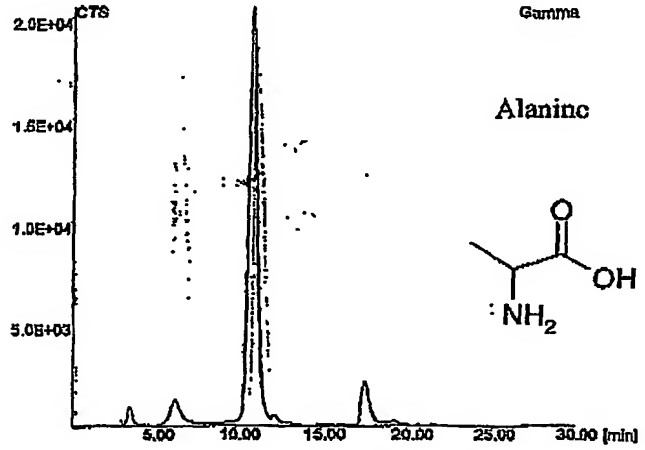
GpG Synthesis and Reaction

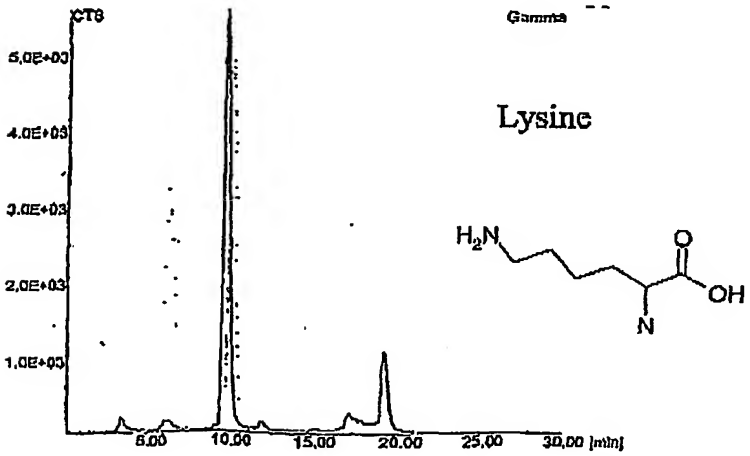
EXAMPLE 10

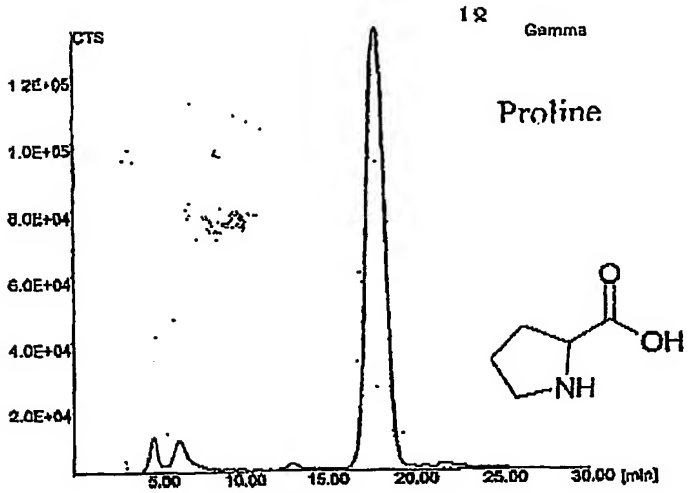
^{99m}Tc Labeling of Purine Bases and Amino Acids

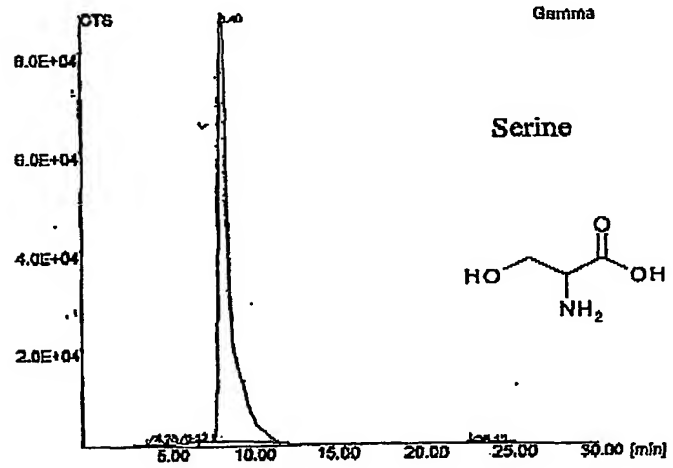
1. Labeling of Amino Acids

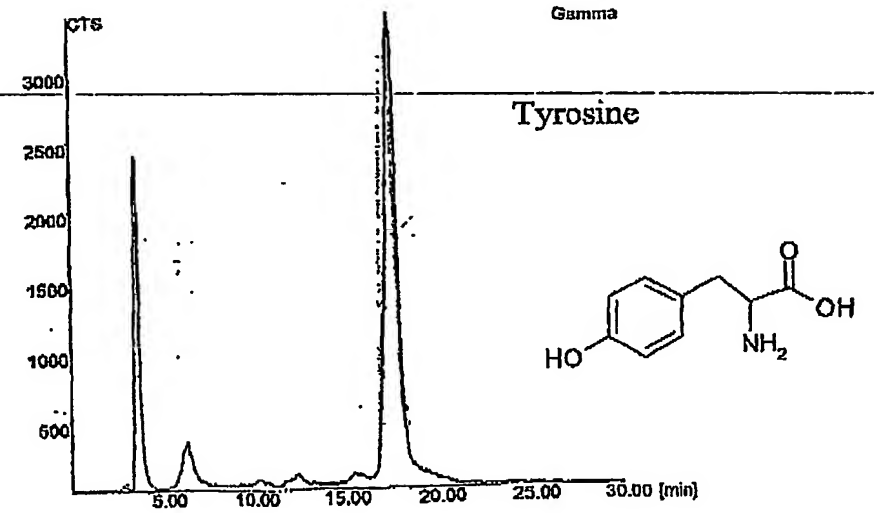
The protocol for labeling amino acids involves a standard method (i.e. neutralization of $^{99m}Tc(CO)_3$ solution with HCl followed by addition of phosphate buffer pH 7.4) in which one requires a final concentration of amino acid of 1mM (in H_2O). Heating the solution to 90°C for 30 min yields 90-98% labeling. Below some representative traceshave been shown. In all cases thus far the RT of the ^{99m}Tc labeled amino acids corresponds to the relative RT of the Re complexes.





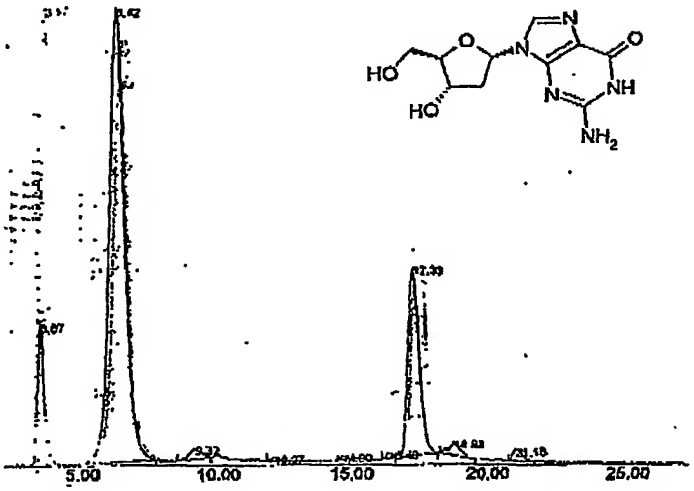

1Q
Gamma
CTS
1.2E+05
1.0E+05
8.0E+04
6.0E+04
4.0E+04
2.0E+04
5.00
10.00
15.00
20.00
25.00
30.00 [min]
Proline
O
OH
NH


Gamma
CTS
8.0E+04
6.0E+04
4.0E+04
2.0E+04
5.00
10.00
15.00
20.00
25.00
30.00 [min]
Serine
O
HO
OH
NH2


Gamma
CTS
3000
2500
2000
1500
1000
500
5.00
10.00
15.00
20.00
25.00
30.00 [min]
Tyrosine
O
OH
HO
NH2

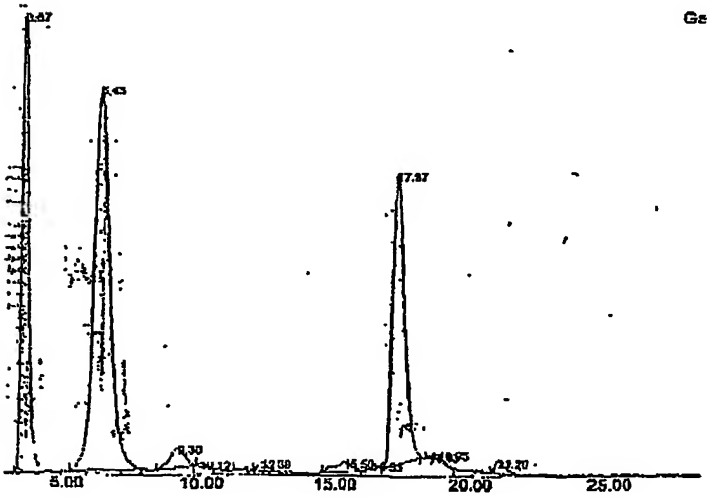
Labeling of Glycine also works fine (results not shown).

2. Labeling of Purine Bases



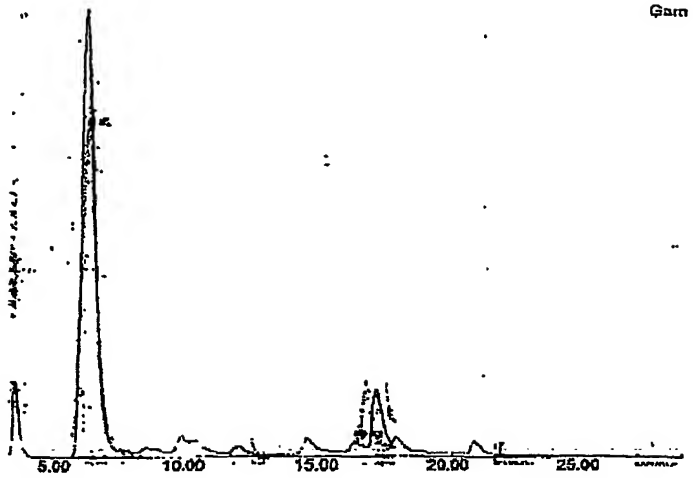
Labeling of 2dG ~1mM, phosphate buffer pH 7.4, 90°C, 1h

15

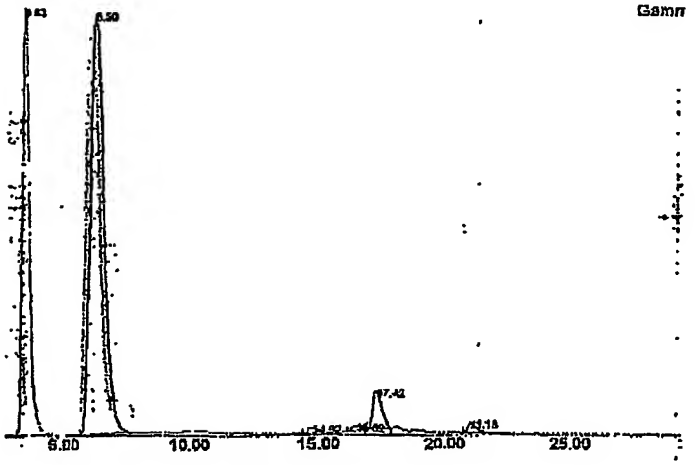


Labeling of 2dG ~1mM, phosphate buffer pH 7.4, 90°C, 3h

25 Labeling of G under the same conditions shows a similar trace. At pH = 6 (acidic conditions) and pH = 8 (basic conditions) there is no labeling after 2h (see below) for either 2dG or G.



10 **Labeling of 2dG ~1mM, pH 6, 90°C, 2h**



20 **Labeling of 2dG ~1mM, pH 8, 90°C, 2h**

CLAIMS

1. Rhenium tricarbonyl compounds as described in the specification.
2. Rhenium tricarbonyl compounds as claimed in claim 1 for use in radiotherapy.